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On the biology, ecology and conservation of *Jordanita (Gregorita) hispanica* (Alberti, 1937) (Lepidoptera: Zygaenidae, Procridinae)

K. A. Efetov & G. M. Tarmann

Abstract

Hitherto, the early stages of *Jordanita (Gregorita) hispanica* (Alberti, 1937) have only been briefly described (EFETOV & TARMANN, 2003). In the present paper a more extended description is given, new data on the biology and ecology are presented and the early stages figured. Moreover, for accurate identification the male and female genitalia and the DNA-barcode are provided. The holotype of this species is figured.

KEY WORDS: Lepidoptera, Zygaenidae, Procridinae, *Jordanita hispanica*, early stages, biology, DNA-barcode, holotype, Spain, Portugal, France.

Sobre la biología, ecología y conservación de *Jordanita (Gregorita) hispanica* (Alberti, 1937)
(Lepidoptera: Zygaenidae, Procridinae)

Resumen

Hasta ahora, los primeros estadios de *Jordanita (Gregorita) hispanica* (Alberti, 1937) solamente han sido descritos brevemente (EFETOV & TARMANN, 2003). En el presente trabajo se da una descripción más extensa, se presentan nuevos datos sobre la biología y la ecología y se dan los primeros estadios. Además, para la identificación exacta, se suministra la genitalia del macho y de la hembra y el ADN-código de barras.

PALABRAS CLAVE: Lepidoptera, Zygaenidae, Procridinae, *Jordanita hispanica*, primeros estadios, biología, AND-código de barras, holotipo, España, Portugal, Francia.

Introduction

The family Zygaenidae is an important model for ecological, environmental, zoogeographic, karyological, biochemical and taxonomical investigations (EFETOV *et al.*, 2004, 2010, 2014b, 2014c, 2015, 2016, 2018; EFETOV & KNYAZEV, 2014; EFETOV & SAVCHUK, 2009; EFETOV & TARMANN, 2017a; KNYAZEV *et al.*, 2015; CAN CENGİZ *et al.*, 2018; CAN *et al.*, 2019; TARMANN, 2019) in Lepidoptera. Its systematics is well established. According to the contemporary classification the family Zygaenidae consists of five subfamilies: Inouelinae Efetov & Tarmann, 2017; Procridinae Boisduval, 1828; Chalcosiinae Walker, 1865; Callizygaeninae Alberti, 1954; and Zygaeninae Latreille, 1809 (EFETOV, 1999, 2001a, 2001b, 2004; EFETOV *et al.*, 2014a; EFETOV & TARMANN, 2017a; TARMANN, 1994). Many species have been recently described (see e. g. EFETOV, 1997a, 1997b, 1998, 2006, 2010; EFETOV & TARMANN, 2013a, 2013b, 2014a, 2014b, 2016a, 2016b, 2017b; MOLLET, 2018; MOLLET & TARMANN, 2018; TARMANN & COCK, 2019; TARMANN & DROUET, 2015). However, even in Western Europe the biology and early stages of some species are poorly

studied or even unknown. A good example is *Jordanita (Gregorita) hispanica* (Alberti, 1937), a species known from Spain, Portugal and France.

The Iberian Peninsula is one of the richest European regions for endemism in the Zygaenidae. Five forester moths (Procridinae), viz. *Rhagades (Wiegelia) predotae* (Naufock, 1930), *Adscita (Adscita) jordani* (Naufock, 1921), *A. (A.) schmidti* (Naufock, 1933), *A. (Tarmannita) bolivari* (Agenjo, 1937), and *Jordanita (Jordanita) vartianae* (Malicky, 1961), and one burnet moth (Zygaeninae), viz. *Zygaena (Agrumenia) ignifera* Korb, 1897, are endemic and one more Procridinae, viz. *Jordanita (Gregorita) hispanica* (Alberti, 1937) and two Zygaeninae, viz. *Zygaena (Mesembrynnus) contaminei* Boisduval, 1834, and *Z. (Zygaena) anthyllidis* Boisduval, 1828, are subendemic to the Iberian Peninsula. This high endemism is based on the exceptional environmental situation here with long isolation and independent development of wildlife during the last glacial periods. Today, the extreme changes of the environment in Spain because of the climatic changes in the last decades – and from other causes – require careful documentation of these highly endangered endemic species. To protect them and to prevent their extinction, intensive field work and monitoring of these environmental changes are required. A profound knowledge of the biology and ecology of endemic species is crucial for this purpose. The authors have repeatedly studied these endemisms. One species, viz. *Jordanita hispanica*, is discussed in this paper.

Materials and methods

The first field studies on *J. hispanica* go back to the year 1978 when a colony was discovered by G. M. T. in the south-western Alps on Mont Ventoux in the Department Vaucluse in southern France. Females were observed ovipositing on *Centaurea paniculata* L. (Asteraceae) (TARMANN, 1992; NAUMANN *et al.*, 1999; EFETOV & TARMANN, 1999). A few larvae were reared from these eggs until L3 on *Centaurea jacea* L. and *C. scabiosa* L. in captivity, but then died. In Spain the observations of G. M. T. started in 1979 in the Provinces Cuenca and Madrid. However, it was not before both authors of this study undertook a joint field trip to Spain in 2002 that a female was found, eggs were obtained and the species could be reared from egg to imago for the first time.

Description of the life history of *Jordanita hispanica*

HABITATS

1. FRANCE, Vaucluse, Mont Ventoux south side, Forêt de la Perache, 1180 m: rocky calcareous plateau (Cretaceous limestone) with Mediterranean steppe vegetation in open *Quercus pubescens* forest.
2. SPAIN, Segovia, east of San Rafael, El Espinar, 1260 m: dry, rocky slope beside open pine forest (Fig. 1).

OVIPOSITION

The first oviposition was observed in nature on 24 July 1978 by G. M. T. at noon (around 12 o'clock, summer time) on the stems below and on the calyx of flowering *Centaurea paniculata* L. (Asteraceae) plants at Forêt de la Perache. A second oviposition was observed on 15 June 2002 by K. A. E. & G. M. T. at El Espinar, again at noon (exactly at 12 o'clock, summer time) on *Centaurea cf. ornata* Wild., again singly and on the calyx of the flower heads. When ovipositing the female bends its abdomen downwards and attaches the eggs singly or in a short row (the eggs not touching each other) on the surface of the plant.

EGG (Fig. 2)

The egg is ovoid, length 0.7 mm, breadth 0.4 mm, height 0.3 mm, and of whitish yellow (EFETOV & TARMANN, 2003) or yellowish green colour (NAUMANN *et al.*, 1999).

LARVA (Figs 3-5)

First instar (L1). Final length before moulting 0.9-1.0 mm, breadth 0.3 mm. Body whitish yellow, head and anal comb brown. The setal formula of the first abdominal segment is: D: 1d, 1l; SD: 0d, 1l; L: 0d, 2l (the terminology of the setae follows EFETOV *et al.* (2006) and EFETOV & HAYASHI (2008); D = dorsal setae, SD = subdorsal setae, L = lateral setae, l = light, hair-like setae, d = dark, stronger sclerotized setae). This setal combination is typical for the subgenera *Gregorita* Povolný & Šmelhaus, 1951, *Tremewanria* Efetov & Tarmann, 1999, and *Jordanita* Verity, 1946, of the genus *Jordanita* (EFETOV, 2001c; EFETOV & TARMANN, 2003). Duration of L1: 7 days.

Second instar (L2). Colour and pattern like first instar. Final length before moulting 2.2 mm, breadth 0.7 mm. Duration of L2: 12 days.

Third instar (L3) (Fig. 3). Final length before moulting 3.3 mm. Dorsolateral area differentiates into stripes and the pattern of the adult larva is already visible. Duration of L3: 15-16 days.

Fourth instar (L4). Final length before moulting 3.5 mm. Mediodorsal line interrupted, forming a row of oval, dark brown spots that are embedded into a broad grey dorsal band; space between mediobursal and lateral area (except dorsal verrucae) greyish yellow; lateral and basal lines absent, lateral area greyish brown dorsally, whitish brown ventrally; ventrolateral area light brown; dorsal verrucae light reddish brown, subdorsal, lateral and subventral verrucae brown; ventral part of larva crimson. Thoracic legs dark brown, abdominal prolegs crimson. Head dark brown; prothoracic plate dark brown with white mediobursal line. Duration of L4 before hibernation: 22-26 days.

Hibernation in L4.

Fifth and sixth instars (L5, L6). Pattern like L4.

Seventh and eighth instars (L7, L8) (Figs 4-5). Final length before moulting of L8 12.5 mm. Mediobursal line interrupted, forming a row of hammer-shaped (T-shaped), dark brown spots that are embedded into a broad grey dorsal band; a visible dorsolateral line is absent; space between mediobursal and lateral area (except dorsal verrucae) whitish yellow; lateral and basal lines absent; lateral area greyish brown dorsally, whitish brown ventrally; ventrolateral area light brown; dorsal verrucae light reddish brown, subdorsal, lateral and subventral verrucae reddish brown; ventral part of larva light red. Thoracic legs dark brown, abdominal prolegs light red. Peritreme of spiracles blackish brown. Head blackish brown; prothoracic plate dark brown with white mediobursal line. Anal comb dark brown, with 12 setae. Except for the verrucae, body covered with sclerotized dark brown multisped macrotubercles. Dorsal verruca of first abdominal segment with 6 long, light setae and 24 short, dark setae. Duration of L8: 10 days.

PUPA (Fig. 6)

Length 7.5 mm. Head, thorax, wings and abdomen smooth, shiny, light brown; proboscis long but not extending beyond the end of the abdomen. Duration of pupal stage: 16 days.

COCOON

Length 12.0 mm, breadth 5.0 mm, spindle-shaped, white, constructed of loosely spun silk.

ADULTS (Figs 7–8)

The imagines emerge in the morning. Activity starts shortly after the sun arrives at the habitat when males and females are looking for flowers to obtain nectar. Copula takes place in early evening and lasts until next morning. The females oviposit during the hottest hours at midday.

The males of this species are found during day and night and are frequently attracted by UV light (DROUET, 2016).

It is also important to mention that during our observations in 2002 *J. hispanica* was frequently occurring sympatrically, syntopically and synchronously with *Jordanita (Tremewanina) notata* (Zeller, 1847). Both species externally are extremely similar and cannot be distinguished in nature even by a specialist. Moreover, *J. notata* is more common than *J. hispanica* in central Spain.

Discussion

J. hispanica is the type-species of the subgenus *Gregorita* Povolný & Šmelhaus, 1951 (by original designation). This subgenus is represented by 8 species of which 7 occur in North Africa. In Europe only *J. hispanica* is present, distributed in Portugal, Spain and France (Fig. 10) (EFETOV & TARMANN, 1999; FERNÁNDEZ-RUBIO, 2005; DROUET, 2016). In the male genitalia in all species of this subgenus the valva is without process and the phallus is characterised by an aedeagus that is broader distally, with a single cornutus on the vesica seminalis (Fig. 11). In the female genitalia of many species of Procridiniae a special structure exists named ‘praebursa’. The praebursa is the strongly dilated distal part of the ductus bursae between the antrum and the corpus bursae (ALBERTI, 1954; EFETOV, 1996; EFETOV & TARMANN, 2014a, 2014b, 2016a). In the subgenus *Gregorita* this structure is present only in some North African species. In *J. hispanica* the female genitalia lack a praebursa (Fig. 12), the proximal part of the ductus bursae is funnel-shaped, smooth, with a slightly grooved structure distally (TARMANN & TREMEWAN, 1995).

The larvae of *Gregorita* are characterised by an inverted T-shaped sclerite (prothoracic plate) on the prothoracic segment, dorsal verrucae that are almost contiguous mediadorsally or mediadorsally fused to form a large, horizontal dorsal ‘spot’ on each segment and the pattern on the 10th abdominal segment forms a characteristic ‘five-spot cluster’ (TARMANN & TREMEWAN, 1995; MOLLET, 2003).

As far as it is known, the females of all *Gregorita* species lay their eggs singly or in short rows and attach them either on the calyx on the flower head, the distal part of the stem or on the leaves of the larval host-plant. The larvae are either leaf-mining until pupation or feed freely after hibernation in the later instars depending of the larval host-plants (TARMANN & TREMEWAN, 1995; MOLLET, 2003).

Identification and additional information

For the conservation of a species it is essential that it can safely be identified. Only then it is possible to observe changes in its population density and to realise a decrease in the number of specimens and populations. Unfortunately, no stage of *J. hispanica* can be identified with certainty by habitus in nature. The early stages as well as the adults have to be collected. In the first case the larvae have to be reared to imago (by entomologists with some experience in Procridiniae rearing) and the genitalia of the imagines have to be studied after dissection of the abdomen and the structures compared with the known literature. Therefore, we provide the necessary information below and include also two DNA-barcodes (EFETOV *et al.*, 2019) for the genetic identification of early stages, adult specimens and parts of specimens of *J. hispanica*.

The holotype of this species (Figs 8-9) is deposited in Zoologische Sammlungen des Bayerischen Staates (ZSBS), Munich, Germany (EFETOV & TARMANN, 1999).

For the identification of the adults we provide the drawings of male and female genitalia of *J. hispanica* (Figs 11-12) and another Spanish species, viz. *J. (Tremewanina) notata* (Zeller, 1847) (Figs 13-14), which is usually flying together with *J. hispanica*, has similar habitus and the same larval host-plant.

DNA barcodes (COI gene sequences) (EFETOV *et al.*, 2019) of two male specimens of *J. hispanica* (SPAIN, Segovia, 1 km SE Navafría, 1300 m, 11-VI-2002, K. A. Efetov & G. M. Tarmann leg.) are listed below.

Genbank number HM386570:

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AACACTTTATTTATTTGGTGTGATCAGGAATAGTTGGTACATTATAAGTGT
AATTCTGTCAGAAATTAGGAGCTCCAGGATCTTAATTGGTGATGATCAAATTATAATA
CTATTGTTACCGCTCATGCTTTATTATAATTTTTTATAGTTACCTATTATAATTGGT
GGATTGGAAATTGATTAGTCCTTAATATTAGGGCTCCAGATATAGCTTCCCACG
AATAAATAATATAAGATTTGACTTCTCCCCCTTCATTAACCTTTAATTCAAGAAG
AATTGTAGAACAGGAGCTGGAACAGGATGAACCGTTACCCCCCTCTTCATCTAAC
ATTACTCATAGAGGGAGGTTCTGTAGATTAGCAATTTCCTTACATTAGCAGGTATT
TCTTCAATTAGGAGCAGTAAATTATCACAACATTATAATATAACGACCTGATGGA
ATATCATTGATCAAATACCTTTATCGTTGAGCAGTGGAAATTACTGCTTTATTATTAT
TGCTTCTTACCTGTATTAGCTGGAGCAATTACTATACTTTAACTGATCGAAATCTTA
ATACATCATTTTGATCCTGCAGGTGGGGAGATCCAATTCTTATCAACATTATTT
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Genbank number HM386571:

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AACACTTTATTTATTTGGTGTGATCAGGAATAGTTGGTACATTATAAGTGT
AATTCTGTCAGAAATTAGGAGCTCCAGGCTCTTAATTGGTGATGATCAAATTATAATA
CTATTGTTACCGCTCATGCTTTATTATAATTTTTTATAGTTACCTATTATAATTGGT
GGATTGGAAATTGATTAGTCCTTAATATTAGGAGCTCCAGATATAGCTTCCCACG
AATAAATAATATAAGATTTGACTTCTCCCCCTTCATTAACCTTTAATTCAAGAAG
AATTGTAGAACAGGAGCTGGAACAGGATGAACCTGTTACCCCCCTCTTCATCTAAC
ATTGCTCATAGAGGGAGGTTCTGTAGATTAGCAATTTCCTTACATTAGCAGGTATT
TCTTCAATTAGGAGCAGTAAATTATCACAACATTATAATATAACGACCTAATGGA
ATATCATTGATCAAATACCTTTATCGTTGAGCAGTAGGAATTACTGCTTTATTATTAT
TGCTTCTTACCTGTATTAGCTGGAGCAATTACTATACTTTAACTGATCGAAATCTTA
ATACATCATTTTGATCCTGCAGGTGGGGAGATCCAATTCTTATCAACATTATTT
```

Habitat preference and conservation

The preferred habitats of *J. hispanica* are unfertilised, dry, sometimes rocky, meadows with a diverse flora that contain good numbers of the respective larval host-plant. Like many Procridinae this species is also dependent on a steady succession in the vegetation, created by natural conditions (e. g. by rock falls, moving scree, heavy winds etc.) or human activities (e. g. by wood cutting, continuous grazing etc.).

In the last years *J. hispanica* has become endangered on the Iberian Peninsula especially due to the dramatic climatic changes and the transformation of former suitable habitats into semideserts. In France several of the former habitats are now overgrown by dense forest (e. g. Forêt de la Perache, Mt. Ventoux).

In general *J. hispanica* should be able to survive these changes by shifting around through suitable habitats as it obviously did in history. However, continuous observations on its changing

status could help to obtain a better overview of potential threats and population changes. For this, continuous field work is necessary. The Scientific Project of SHILAP on the Iberian Peninsula and the work of GIRAZ in France where experts monitor the population changes of Lepidoptera (including Zygaenidae) are good bases for preserving this rare species.

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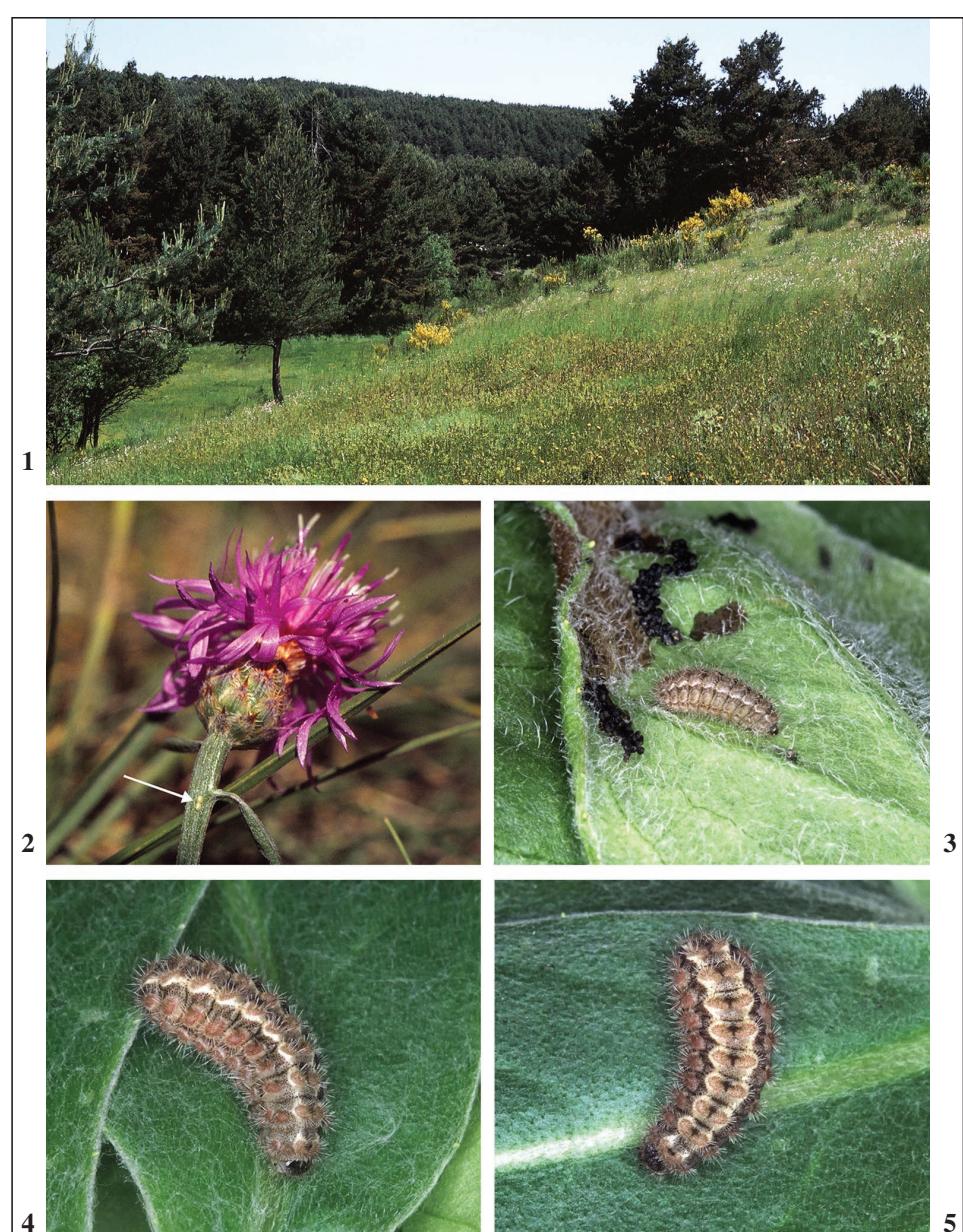
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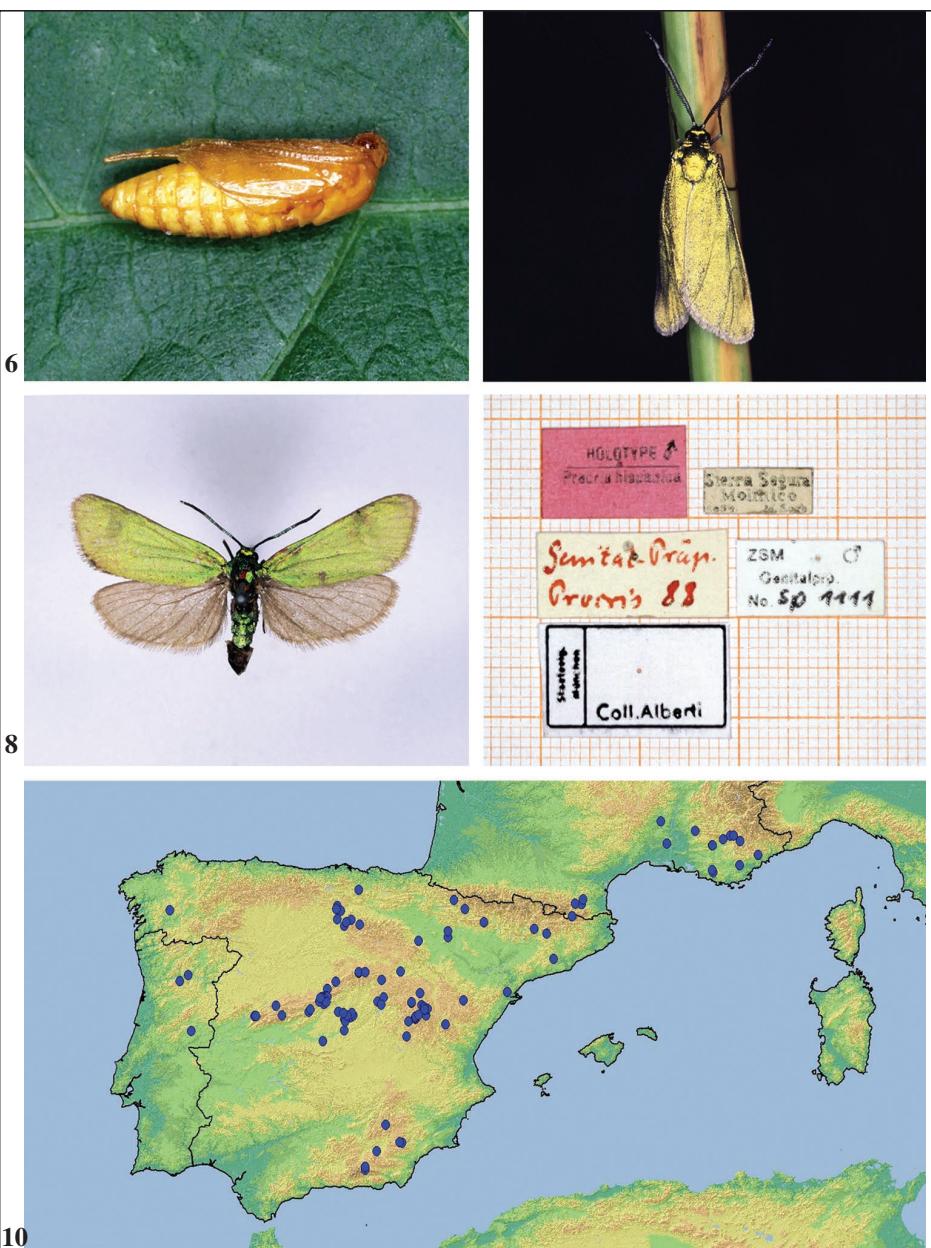
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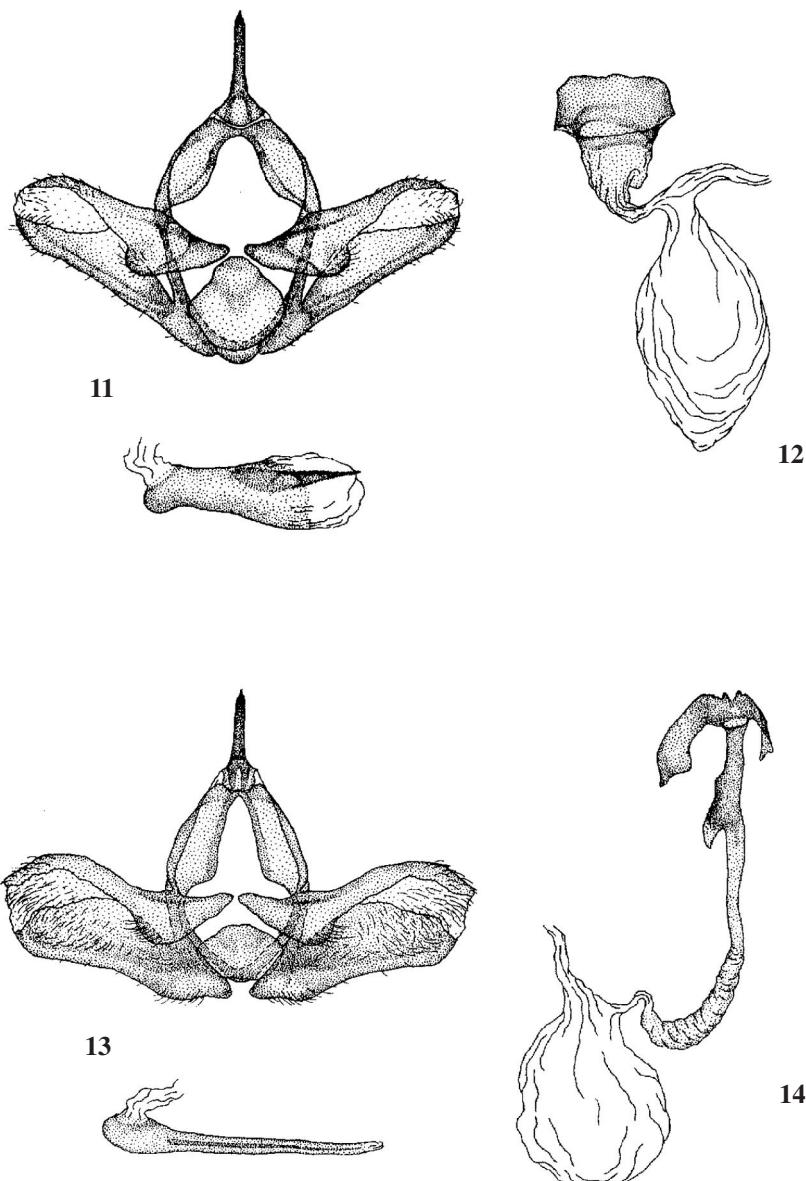
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Figures 1-5.- **1.** Habitat of *J. hispanica* at Spain, Segovia, east of San Rafael, El Espinar. **2.** Eggs (shown by arrow) on the stem below the flower of *Centaurea paniculata* L. at France, Mt. Ventoux, Forêt de la Perache. **3.** Third instar larva of *J. hispanica* on the leaf of *Centaurea montana* L. (in captivity). **4.** Adult (L8) larva of *J. hispanica* on *Centaurea montana* leaf (in captivity) (dorsolateral view). **5.** Adult (L8) larva of *J. hispanica* on *Centaurea montana* leaf (in captivity) (dorsal view).



Figures 6-10.- 6. Pupa of *J. hispanica* (lateral view). 7. Freshly emerged male of *J. hispanica*, reared from egg from San Rafael, El Espinar, Spain. 8. Holotype male of *Procris hispanica* (ZSBS, Munich). 9. Pin-labels of holotype male of *Procris hispanica*. 10. Distribution map of *J. hispanica*. BioOffice database of Tiroler Landesmuseen, Ferdinandeum, Innsbruck, Austria.



Figures 11-14.- 11-12. *Jordanita (Gregorita) hispanica* (Alberti, 1937). 11. Male genitalia, 12. Female genitalia. 13-14. *Jordanita (Tremewania) notata* (Zeller, 1847). 13. Male genitalia, 14. Female genitalia. (Ex EFETOV, 2001c).